APPENDIX A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of DIKSTEIN et al

Serial No. 09/763,909 Group Art Unit: 1642

Filed: June 8, 2001 Examiner: Davis, Minh Tam B

For: **A TRANSCRIPTION FACTOR TFIID SUBUNIT...**

DECLARATION
under Rule 132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Rivka Dikstein, an Israeli citizen residing at 6 Aharonovich Yosef St., Rehovot, Israel, hereby declare:

1. I am one of the inventors of the above captioned application (hereinafter *"the application"*).

2. I am currently a senior scientist in the Department of Biological Chemistry at the Weizmann Institute of Science of Rehovot, Israel.

3. My list of publications is attached herewith as **Annex A**. My fields of expertise include mechanisms of transcription regulation by TBP-associated factors (TAFs) and NF-κB.

4. I have read and am familiar with the contents of the application. I have also read the examiner's objections in the Office action dated March 27, 2003 (hereinafter *"the action"*). I wish to comment on certain remarks made by the examiner in the action.

5. On pages 6 and 7 of the action, the examiner states that one cannot extrapolate the teaching of the specification to the scope of the claims, and that it is unpredictable that full length $TAF_{II}105$ polypeptide mediates the activation of anti-apoptosis genes by NF-κB. I disagree.

6. In the application, we provide evidence that not only exogenously expressed $TAF_{II}105$ is involved in NF-κB mediated anti-apoptotic gene activation but also the native endogenous $TAF_{II}105$ protein. The involvement of the endogenous $TAF_{II}105$ gene in activation of anti-apoptotic genes by NF-κB was shown by knocking down

the levels of endogenous $TAF_{II}105$ using anti-sense expression (see Fig. 8 of the application and pg. 19, lines 16-27). As a consequence enhanced apoptosis was clearly observed following treatment with the TNFα cytokine, a result that is consistent with the notion that endogenous $TAF_{II}105$ is required for anti-apoptotic gene activation. This experiment together with the experiments of exogenously expressed $TAF_{II}105$ proteins, wild type and mutants, and the biochemical data, have been accepted by all the three expert reviewers of our article Yamit-Hezi, A. and Dikstein, R. (1998). $TAF_{II}105$ Mediates Activation of Anti-Apoptotic Genes by NF-κB. EMBO J. 17, 5161-5169 (**Appendix 3**), as evidence that $TAF_{II}105$ is required for activation of anti-apoptotic genes.

7. The examiner mentions several times in his remarks that the polypeptides increase or decrease the basal activity of NF-κB. I wish to point out that the results presented in the application relate to the induced activity of NF-κB, and not the basal activity. I refer the examiner to Figure 5a to 5d, and to Figure 6c. I also wish to point out that p65 is a subunit of the NF-κB transcription factor and thus it represents NF-κB. The anti-apoptotic activity of NF-kB is conferred by p65. I refer the examiner to Van Antwerp et al., 1996 (**Appendix 7**) and Beg and Baltimore, 1996 (**Appendix 8**).

8. On pages 9-15 of the action, the examiner states that one cannot extrapolate the teaching of the specification to the scope of the claims, and that there is no correlation between the *in vitro* induction of apoptosis described in the specification and the claimed *in vivo* treatment of cancer. I disagree.

9. For treating cancer, the goal is to achieve death of the cancerous cell. It doesn't matter if such a treatment involves an unnatural concentration of a compound that can promote apoptosis, such as $TAF_{II}105\Delta C$. The idea is to shift the balance between survival and death towards death (apoptosis).

10. We recently published a study in which we examined the effect of $TAF_{II}105\Delta C$ in a transgenic mice model system *in vivo*, and obtained results consistent with those *in vitro*, namely that this protein (at very low concentrations) inhibits transcription activation of NF-κB dependent anti-apoptotic genes (Silkov, A. Wolstein, O., Shachar, I and Dikstein, R. 2002. *Enhanced apoptosis of B and T lymphocytes in $TAF_{II}105$ Dominant Negative Transgenic Mice is Linked to NF-κB.* J. Biol. Chem 277, 17821-17829 (**Appendix 2**)).

11. I would also like to point out that the cells used in the experiments described in the application (293 and HeLa cell lines) are standard, internationally recognized models for developing *in vivo* cancer treatments.

12. Another example of an *in vitro* study that is confirmed *in vivo* can be found in the following articles. The involvement of NF-κB in anti-apoptotc gene activation in response the the TNFα cytokine is shown *in vitro* (Van Antwerp, D.J., Martin, S.J., Kafri, T., Green, D.R. and Verma I.M. 1996. *Suppression of TNF-alpha-induced apoptosis by NF-kappaB*. Science **274**: 787-789 (**Appendix 7**)), and is confirmed *in vitro* and *in vivo* in the article: Beg, A.A. and Baltimore, D. 1996. *An essential role for NF-κB in preventing TNF-α-induced cell death.* Science **274**: 782-784 (**Appendix 8**).

13. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____ _____

Dr. Rivka Dikstein

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11. I would also like to point out that the cells used in the experiments described in the application (293 and HeLa cell lines) are standard, internationally recognized models for developing *in vivo* cancer treatments.

12. Another example of an *in vitro* study that is confirmed *in vivo* can be found in the following articles. The involvement of NF-κB in anti-apoptotc gene activation in response the the TNFα cytokine is shown *in vitro* (Van Antwerp, D.J., Martin, S.J., Kafri, T., Green, D.R. and Verma I.M. 1996. *Suppression of TNF-alpha-induced apoptosis by NF-kappaB*. Science 274: 787-789 (**Annex B**)), and is confirmed *in vitro* and *in vivo* in the article: Beg, A.A. and Baltimore, D. 1996. *An essential role for NF-κB in preventing TNF-α-induced cell death*. Science 274: 782-784 (**Annex C**).

13. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 17, 2003

Rivka Dikstein

Dr. Rivka Dikstein

Curriculum Vitae

A. Personal details

Date and place of birth:	12.02.1961
Immigration to Israel:	June 1963
Marital status:	Married, 3 children
Citizenship:	Israeli
Mailing Address:	Dept. of Biological Chemistry The Weizmann Institute of Science Rehovot 76100 Israel
Contact information:	Phone: 972-8-9342117 Fax: 972-8-9344118 E-mail: rivka.dikstein@weizmann.ac.il

B. Education

1993-1996:	Post doctoral training at the University of California, Berkeley, under the supervision of Dr. Robert Tjian. Research topic: Characterization and cloning of TFIID subunits.
1988-1993:	Ph.D. studies at The Weizmann Institute of Science, Dept. of Molecular Genetics under the supervision of Dr. Yosef Shaul. Thesis: Characterization of EP binding protein, an activator of Hepatitis B virus enhancer.
1985-1988:	M.Sc. studies at The Weizmann Institute of Science, Dept. of Virology under the supervision of Dr. Yosef Shaul. Thesis: Multiple functional elements activate the Hepatitis B virus enhancer.
1982-1985:	Tel Aviv University, Israel, Faculty of Life Sciences B.Sc. degree

C. Employment History

1997-present:	Senior Scientist, The Weizmann Institute of Science, Dept. of Biochemistry.

D. Teaching Experie

1997-1999:	Lab course: Basic methods in molecular biology
1999:	Course of lectures: Mechanisms of transcription

E. International Recognition

Awards

1993:	John P. Kenedy prize for Ph.D. excellence
1993:	EMBO long term fellowship award
1993:	Rothschild fellowship award (declined)
1993:	Fulbright fellowship award (declined)
1997:	Research Career Academy Award by the Israel Cancer Research Foundation
1999:	L. Naftali Science Foundation award
2000:	Jakubskind-Cymerman award

List of publications

Refereed articles

1) Honigwachs, J., Faktor, O., **Dikstein, R.,** Shaul, Y., and Laub, O. (1989). The Liver Specific Expression of Hepatitis B virus is determined by the Combined Action of the Core Gene Promoter and the Enhancer. *J. Virol. 63,* 919-924.

2) **Dikstein R.,** Faktor, O., Ben-Levy, R., and Shaul, Y. (1990). Functional Organization of the Hepatitis B virus Enhancer. *Mol. Cell. Biol. 10,* 3683-3689.

3) **Dikstein R.,** Faktor, O., and Shaul, Y. (1990). Hierarchic and Cooperative Binding of the Rat Liver Nuclear Protein C/EBP at the Hepatitis B Virus Enhancer. *Mol. Cell. Biol. 10,* 4427-4430.

4) **Dikstein, R.,** Heffetz, D., Ben-Neriah, Y. and Shaul Y. (1992). c-Abl has a Sequence Specific Enhancer Binding Activity. *Cell 69*, 751-757.

5) **Dikstein, R.**, Agami, R., Heffetz, D., and Shaul Y. (1996). p140/c-abl that Binds DNA is Preferentially Phosphorylated on Tyrosine Residues. *Proc. Natl. Acad. Sci. USA 93*, 2387-2391.

6) **Dikstein R.,** Ruppert, S., and Tjian, R. (1996). TAF$_{II}$250 is a Bipartite Protein Kinase that Phosphorylates the Basal Transcription Factor RAP74. *Cell 84*, 781-790.

7) **Dikstein R.,** Zhou, S., and Tjian, R., (1996). Human TAF$_{II}$105 is a Cell Type Specific TFIID Subunit Related to hTAF$_{II}$130. *Cell 87*,137-146.

8) Yamit-Hezi, A. and **Dikstein, R.** (1998). TAF$_{II}$105 Mediates Activation of Anti-Apoptotic Genes by NF-κB. *EMBO J. 17,* 5161-5169.

9) Wolstein, O., Silkov, A., Revach, M. and **Dikstein, R.** (2000). Specific Interaction of TAF$_{II}$105 with OCA-B is involved in Activation of Octamer-Dependent Transcription. *J. Biol. Chem 275, 16459-16465.*

10) Yamit-Hezi, A., Nir, S., Wolstein, O. and **Dikstein, R.** (2000). Interaction of TAF$_{II}$105 with Selected p65/RelA Dimers is Associated with Activation of Subset of NF-κB Genes. *J. Biol. Chem 275, 18180-18187.*

11) Matza, D., Wolstein, O., **Dikstein, R.**, and Shachar, I. (2001). Invariant Chain Induces B Cell Maturation by Activating TAF$_{II}$105-NF-κB Transcription Program. *J Biol Chem.* 276, 27203-27206.

12) Rashevsky-Finkel, A., Silkov, A. and **Dikstein, R.** (2001). A Composite Nuclear Export Signal in the TBP-Associated Factor TAF$_{II}$105, *J. Biol. Chem.* 276, 44963-44969.

13) Torchinsky, A., Li[redacted]nski, L., Wolstein, O., Shepshelovich[redacted]., Savion, S., Orenstein, H., Zaslavsky, Z., Carp, H., Brill, A., **Dikstein, R.**, Toder, V., Fein, A. (2002). NF-κB DNA-Binding Activity in Embryos Responding to a Teratogen, Cyclophosphamide. *BMC Developmental Biology* 2, 2.

14) Silkov, A. Wolstein, O., Shachar, I and **Dikstein, R.** (2002). Enhanced apoptosis of B and T lymphocytes in $TAF_{II}105$ Dominant Negative Transgenic Mice is Linked to NF-□appaB. *J. Biol. Chem* 277, 17281-17829.

15) Ainbinder, E., Revach, M., Wolstein, O., Moshonov, S., **Dikstein, R.** (2002) The Mechanism of Rapid Transcriptional Induction of TNF-Alpha Responsive Genes by NF-kappaB. *Mol. Cell. Biol.* 22, 6354-6362.

Book Chapters

16) **Dikstein, R.** (2002). TATA box, TBP and TAF. Encyclopedia of Molecular Medicine.